Yoghurt Production by Lactobacillus Fermentation of Soybean Milk

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A simple and inexpensive method of treating soybean to improve the flavour of soybean milk is described. Fermentation of this milk *L. acidophilus* strains and subsequent flavouring notably with lemon flavour has yielded an acceptable yoghurt. This yoghurt can be stored at 5°C for up to 19 days without significant change.

SOYBEANS rich in protein have been an important food item in Asia for centuries. In the 20th century, even in Europe and the United States, soybeans have become increasingly popular as a protein source to replace meat and dairy products. However, the use of soybeans as food is still limited, partly because of their objectionable flavour and also because they contribute flatulence. Numerous methods, therefore, have been developed either to remove or to reduce these undesirable factors.

A large variety of compounds that may contribute to the flavour of soybean products have been isolated, but specific compounds responsible for the flavour have not been identified. One of the theories is that some undesirable flavours are not originally present in the beans, but when the beans are cracked or damaged in the presence of moisture and air, rancid and off-flavours develop. Wilkens *et al*[1] observed that soybean milk prepared from full-fat soybeans was described as beany, rancid, and off-flavoured, whereas soybean milk from defatted beans had a comparatively bland cereal-type odour. They postulated that off-flavours are due to the oxidation of polyunsaturated fats by lipoxygenase. Mustakas *et al*[2] also concluded that inactivation of lipoxygenase is an important step in the preparation of bland-flavoured full-fat soybean flour. Several methods have since been proposed to inactivate the enzyme. Wilkens *et al*[1] suggested a high-temperature, rapid-hydration grinding process for making a nearly bland soybean milk. Mustakas *et al*[3] proposed a dry-heat preconditioning step to inactivate lipoxygenase and other enzymes. Nelson *et al*[4] recommended blanching in boiling water before grinding. Because lipoxygenase is inactive at low pH, Kon *et al*[5] ground soybeans at pH below 3.85 and then cooked the slurry to destroy the enzyme. Mustakas[6] also prepared a lipid-protein concentrate for beverages from full-fat soybean meal by cooking under an acidic condition.

Fermentation processes, which may induce better flavour, destroy or mask disagreeable ones, have long been used in the Orient to improve the palatibility of soybean products such as tempeh, soysauce, miso, and sufu. Although soybean milk has a long history as a popular beverage in the Orient, it was not until the late 1960's that investigators began to develop fermented products from soybean milk by using the same cultures and processes employed by the dairy industry. According to Obara[7] of Tokyo University of Education, Japan, no acceptable product can be obtained from soybean milk by the conventional cheese-making process, and he suggested treating soybean curd with protease followed by fermentation with *Streptococcus cremoris* and *S. lactis* (1:1). Microorganisms do behave differently on different substrates, and they vary greatly among different strains of the same species, as well as between different species. Therefore, it is not surprising that cultures and processes used in the dairy industry are often unsuited for soybean milk fermentation.

Wang *et al*[8] examined eight strains of *Lactobacillus acidophilus* and four strains of *L. bulgaricus* for their ability to ferment soybean milk. They found that *L. acidophilus* strain NRRL B-1910 consistently produced a better product than any of the other cultures studied. Furthermore, the beany flavour of fermented soybean milk was greatly reduced as compared to unfermented soybean milk. In 1975, Stern *et al*[9] investigated the utilization of various sugars by the same cultures that Wang *et al* studied, and found that *L. acidophilus* strains NRRL B-1910 and B-1911 were most effective in using stachyose and raffinose as carbon sources; presence of these sugars in soybeans has been suggested as the cause of flatulence. *L. acidophilus* NRRL B-2092 used sucrose well, and it also gave a good flavour when used to ferment soybean milk. Thus, a mixed strain fermentation of the same species, such as strains NRRL B-1910 and B-2092 of *L. acidophilus*, could lead to a good flavoured food having a low flatulence factor.

Our purpose was first to develop a simple and inexpensive treatment for soybeans to improve the flavour of the milk, and then to use a mixed culture fermentation to make a yogurt-like food.

Traditional soybean milk

Traditionally, soybean milk is a simple water extract of whole soybeans. The beans are soaked in water overnight and ground wet. The slurry is then brought to a boil and filtered to remove the insoluble residue. In some parts of the Orient, beans are soaked in sodium bicarbonate solution, because the natives believe that this treatment reduces the beany flavour. The resultant soybean milk is a stable oil emulsion and has a characteristic flavour, which is unacceptable to many consumers.

Experimental soybean milk

Accepting the concept that heat inactivates enzymes and the belief that soaking in sodium bicarbonate solution reduces the beany flavour, we incorporated both treatments in our process. The beans were soaked in water for 16 hours at 20°C and drained. The soaked beans were boiled in sodium bicarbonate solutions of various concentrations for five minutes and again drained. The bicarbonate-heat-treated beans were then blended two minutes in a *Waring Blendor*[1] with the addition of enough water to give a water to dry beans ratio of 8:1 on a weight basis.

Heat treatment, however, has long been recognised as a method of reducing the solubility of soybean protein. We found that the protein content of soybean milk made from beans boiled in water for five minutes before blending was 1.43% as compared with 3.45% in milk made from beans without boiling. Recently, Wang[10] introduced an ultrasonic procedure to increase the extractability of heat-denatured soybean protein. The blended soybean slurry, therefore, was subjected to ultrasonic treatment for ten minutes at a frequency of 20 kHz with *Sonifier J-32A* of Branson Instruments, Inc. The slurry was centrifuged at 3,000 rpm for five minutes after treatment.

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Table 1. Effect of boiling soybeans in sodium bicarbonate and ultrasonic treatment on protein content and pH of resultant soybean milk.

	Before ultrasonic treatment		After ultrasonic treatment	
Sodium bicarbonate (%)	*Protein (%)*	*pH*	*Protein (%)*	*pH*
0	1.43	6.6	3.44	6.6
0.2	1.53	6.8	3.78	6.8
0.5	1.61	7.1	3.95	7.1
1.0	1.73	7.4	3.96	7.4

To illustrate the effect of sodium bicarbonate and ultrasonication on protein content and pH of the resultant milk, soaked soybeans were boiled for five minutes in various concentrations of sodium bicarbonate solutions before blending. Soluble protein was then determined before and after ultrasonic treatment by a Kjeldahl digestion method based on 6.25 as a conversion factor for nitrogen to protein. As indicated in Table 1, increasing the concentration of bicarbonate from 0 to 1% slightly increased solubility of heat-denatured protein, and also pH of the resultant milk. Ultarsonic treatment, on the other hand, greatly increased the amount of extractable protein. Based on protein content and pH of the resultant milk, we selected 0.2% sodium bicarbonate as the optimal solution for preparing soybean milk. The complete process developed in our laboratory is shown in Fig 1.

Dry soybeans (100 g)
↓
Soaked in water, 16 hours, 20°C
↓
Drained
↓
Boiled in 0.2% $NaHCO_3$ (1,000 ml), 5 minutes
↓
Drained, washed with water
↓
Blended with water (660–680 ml), 2 minutes
(Based on water to dry bean, 8:1)
↓
Ultrasonic treatment 7–10 minutes
↓
Centrifuged, 3,000 rpm, 5 minutes
↓
Experimental soybean milk (about 500 ml)

Fig 1 Process steps in making experimental soybean milk.

Samples of traditional soybean milk and experimental soybean milk made from bicarbonate-heat-treated soybeans were evaluated by 14 members of a flavour panel. Panel members were instructed to describe the predominant flavour for each sample and to assess overall flavour intensity by assigning values from 1 to 10. A score of 1 represented repulsive flavour; 10 representing excellent. Statistical differences between samples were determined by a two-way analysis of variance. Flavour scores and descriptions (Table 2) indicated a significantly higher score on the flavour of bicarbonate-heat-treated soybean milk than that of traditional soybean milk (5.6 vs 3.3). The soybean milk made by our process has improved in flavour, as well as in protein content.

Yogurt from experimental soybean milk

Mixed-strain starter cultures

L. acidophilus strains NRRL B-1910 and B-2092 were used for fermentation. Lyophilized cultures obtained from the ARS Culture Collection maintained at the Northern Laboratory were first cultivated in deep liver medium, and then transferred to soybean milk medium. Each culture was incubated at 37°C for 24 hours and was serially transferred at least twice in the same medium before using as a starter. These cultures were grown singly and blended in the ratio of 1:1 just before their addition to soybean milk medium for yogurt fermentation.

Soybean milk medium

Although sucrose does not significantly affect the growth of *L. acidophilus* B-1910 and B-2092, 5% sucrose was added to the soybean milk to give a balanced taste of sweetness and sourness to the fermented product. Sucrose-enriched soybean milk, however, still is low in titratable acidity after 24 hours at 37°C in a mixed culture fermentation (Table 3). To stimulate bacterial growth which, in turn, would increase titratable acidity, Cheddar cheese whey solids[11] were added to the milk. As whey solids increased from 0 to 4%, the titratable acidity of the resultant yogurt increased from 0.40 to 0.74%. Texture of the yogurt curd also improved from weak to firm as the concentration of whey solids was increased. However, when the amount of whey solids approached 4%, the whey-enriched soybean milk coagulated during sterilization. Consequently, texture of the fermented product was unacceptable. An acceptable soybean milk medium finally selected for further study consisted of 5% sucrose and 2% whey solids.

Sterilization conditions

Soybean milk must be subjected to high-temperature treatment before fermentation to obtain a good nutritional value, as well as to destroy indigenous bacterial spores. However, high temperatures could also have adverse effects on the quality of the fermented product. Hence, it is essential to find an optimum sterilization or pasteurization condition. Data in Table 4 reveal that yogurt made from soybean milk sterilized at 90°C had desirable colour, taste, and titratable acidity, but very soft texture. In addition to a slightly bitter

Table 2. Flavour and odour evaluation of traditional and experimental soybean milks

Evaluation	*Traditional*	*Experimental*
Flavour score*	3.3	5.6**
Descriptions	Rancid	Cereal
	Grassy	Nutty
	Beany	Milky

*A 14-member flavour panel scored for intensity on a 10-point scale where 1 is repulsive and 10 is excellent.
**Significant difference at 99% confidence level.

Table 3. Titratable acidity and texture of soybean yogurt as affected by the addition of cheese whey solids.

Cheese whey solids (%)	*Titratable acidity (% as lactic acid)*	*Texture of curd*
0	0.40	Weak
0.5	0.51	Weak
1.0	0.61	Weak
2.0	0.68	Firm
3.0	0.70	Firm
4.0	0.74	Defective

Table 4. Characteristics of soybean yogurt from soybean milk subjected to varied heat treatment.

Treatment					
Temperature (°C)	*Time (min)*	*Colour*	*Hardness* (g/cm^2)*	*Acidity) (%)*	*Flavour*
90	10	Eggshell white	5	0.71	Good
90	20	Eggshell white	7	0.70	Good
100	10	Eggshell white	13	0.70	Good
100	20	Eggshell white	14	0.70	Good
121	10	Brownish	16	0.78	Slightly bitter
121	20	Brownish	19	0.82	Slightly bitter

*Tested with a curd meter manufactured by Iio Electric Co of Japan.

Table 5. Yogurt quality as affected by protein content of soybean milk.

Extraction ratio (Water:bean)	*Protein (%)*	*Acidity (%)*	*Hardness (g/cm^2)*
6:1	4.5	0.86	17
8:1	3.6	0.71	14
10:1	2.8	0.65	7

taste, yogurt made from soybean milk sterilized at 121°C had a darker colour and harder texture. A satisfactory product, however, can be obtained from soybean milk heated at 100°C for either 10 or 20 minutes. To ensure aseptic conditions, we recommend heating soybean milk at 100°C for 20 minutes.

Effect of protein content of soybean milk on yogurt

Protein content of soybean milk can be regulated by varying the proportion of water to dry beans during extraction. The characteristics of yogurt made from soybean milk with protein contents from 2.8 to 4.5% were investigated. It is evident that the protein content of soybean milk, indeed, affects the acidity and texture of the yogurt produced (Table 5). Yogurt made from soybean milk containing 3.6 and 4.5% protein had a desirable texture, whereas the texture of yogurt made from soybean milk with a low protein content was too soft. Acidity of the yogurt also decreased as the protein of soybean milk decreased. Based on both acidity and hardness, acceptable yogurt can be made from soybean milk containing 3.6 to 4.5% protein.

Gelatin as stabilizer

To prevent whey separation in yogurt, gelatin was used as a stabilizer. Excess amount of gelatin, however, results in a heavy bodied yogurt. To determine the optimal amount of gelatin to use, we incorporated 0.5-1.5% of gelatin into soybean milk at three different protein levels. Adding gelatin prevented free whey; soybean milk with low-protein levels requiring more gelatin than that with high-protein levels. Gelatin needed to prevent free whey and heavy body in soybean milk containing 4.5, 3.6, and 2.8% protein was 0.5, 1.0, and 1.5% respectively.

Flavouring

Such flavours as vanilla, orange, strawberry, or lemon, if desired, can be added to soybean milk before inoculation. We found that lemon flavour is extremely complementary to soybean yogurt.

Our preliminary studies led us to the development of a process to produce soybean yogurt (Fig 2).

Taste panel evaluation

Unflavoured yogurt samples made by the process illustrated in Fig 2 from both traditional and experimental soybean milks were evaluated by the flavour panel. Again, members gave higher quality rating scores to yogurt made from experimental soybean milk than that from traditional milk (6.0 vs 4.5) on a 10-point scale where 1 is repulsive and 10 is excellent. The scores also indicated that fermentation improved the flavour of soybean milk, since the scores for unfermented milk samples were 5.6 and 3.3, respectively.

Keeping quality of soybean yogurt

Texture, taste, and colour are

Gelatin solution, 20%
Autoclaved, 121°C, 10 minutes
1% (24 ml)

Soybean milk (500 ml)
Whey solids (10 g)
Sucrose (25 g)
Steamed, 100°C, 20 minutes
Cooled quickly —0.8%→ Lemon flavour (if desired)
Inoculated, 5% mixed starter
(L. acidophilus B-1910 1
L. acidophilus B-2092 1)
Incubated, 37°C, 24 hours
Soybean yogurt

Fig 2. Outline for yoghurt production from soybean milk.

important keeping qualities of any food. In the case of yogurt, viable bacterial count is also critical, because many yogurt consumers, especially in Japan and Europe, consider the presence of active lactic acid bacteria an essential feature of yogurt. As indicated in Table 6, soybean yogurt can be kept at 5°C for about 19 days without any significant change in acidity, pH, and viable cell count. After that time, our study showed that the viable count was reduced to a point that many yogurt consumers might consider too low. Although the viable cell count of yogurt remained high after about 19 days at 20°C, titratable acidity of the yogurt was too high to suit most consumers' taste. We found that the most acceptable titratable acidity was 0.8% of lactic acid. When the acidity was higher than 0.9%, the yogurt had a harsh flavour.

REFERENCES

1. Wilkens, W. F., Mattick, L. R. and Hand, D. B., *Food Technol.*, 1967, *21*, 1630.
2. Mustakas, G. C., Albrecht, W. J., McGhee, J. E., Black, L. T., Bookwalter, G. N., and Griffith, E. L., *J. Am. Oil Chem. Soc.*, 1969, *46*, 623.
3. Mustakas, G. C., Albrecht, W. J., Bookwalter, G. N., McGhee, J. E., Kwolek, W. F., and Griffith, E. L., Jr., *Food Technol.*, 1970, *24*, 1290.
4. Nelson, A. I., Wei, L. S. and Steinberg, M. P., *Soybean Dig.*, 1971, *31*, 32.
5. Kon, S., Wagner, J. R., Guadagni, D. G., and Horvat, R. J., *J. Food Sci.*, 1970, *35*, 343.
6. Mustakas, G. C., *Cereal Sci. Today*, 1974, *19*, 62.

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Table 6. Keeping quality of soybean yogurt

Temperature (°C)	*Days* 0	4	8	11	15	19	40
				Viable cell count			
5	9×10^8	5.7×10^8	2.2×10^8	4.5×10^7	2.8×10^7	1.1×10^7	9.6×10^4
10	9×10^8	5.7×10^8	3.2×10^8	7.5×10^7	2.8×10^7	8.0×10^6	9.0×10^2
20	9×10^8	1.1×10^9	5.7×10^8	6.4×10^7	4.2×10^7	1.0×10^8	—
				Titratable acidity (% at lactic acid)			
5	0.71	0.70	0.70	0.70	0.70	0.71	0.70
10	0.71	0.72	0.78	0.83	0.77	0.79	0.80
20	0.71	0.87	0.99	1.07	1.16	1.07	1.08
				pH			
5	4.2	4.2	4.2	4.2	4.2	4.2	4.2
10	4.2	4.2	4.0	4.0	4.0	4.0	4.0
20	4.2	4.0	3.9	3.8	3.8	3.8	3.8

7. Obara, T., USDA Final Tech. Rept., Public Law 480, Project UR-All-(40)-26, sponsored by the Northern Regional Research Laboratory, 1968.
8. Wang, H. L., Kraidej, L., and Hesseltine, C. W., *J. Milk Food Technol.*, 1974, *37*, 71.
9. Stern, N., Wang, H. L., and Hesseltine, C. W., Unpublished data, 1975.
10. Wang, L. C., *J. Food Sci.*, 1975, *40*, 549.

Footnotes

[i]The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

[ii]Cheese whey solids were obtained from Dairyland Food Laboratories, Inc., Waukesha, Wis. 53186.